Round-table discussion

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O. Radostits: Perhaps I can pose a question related to why we came here. Do we have sufficient evidence to show that the use of fluoroquinolones in poultry has an effect on *Campylobacter*, and what is the potential human health hazard?

E. Gonder: Is the resistance transferred to humans? This is what we do not know.

O. Radostits: My interest in this topic goes back a long way, almost to the Swann Report, and I have been following the literature since then. My specific interest is the potential of transference of resistance in beef cattle pathogens to humans, because we use antibiotics in beef cattle practice, beef cattle health, and production management. I think that there are eight or nine theoretical steps from the presence of the organism in the animals, to the use of the antibiotics and the emergence of resistance and slaughtering of the cattle. Beef is not sterile when it is fresh; are the organisms transferable to humans working on the farm, in the factory plant, in the retail stores, and at the consumer level, and does that organism colonize those humans? Does it cause disease in those humans? Is it difficult to treat? I have been playing around with a model. I am not a statistician, nor a mathematician, and am trying to estimate the probabilities in each one of those steps and multiply them.

Question: *What evidence do we have that this link exists between the use of fluoroquinolones in poultry and the subsequent dire consequences in humans? Will we ever be able to reconstruct that? I think that Dr Wassenaar mentioned in her paper that Endtz from Holland says it would be impossible to reconstruct that link.*

T. Wassenaar: If you had good genotyping evidence that it did actually occur, you should be able to follow bad bugs from chicken to people, if you could fingerprint them. My own analysis, which doesn't have the luxury of following individual campylobacters or strains, suggests to me that if chickens are not significantly or detectably carrying *Campylobacter* to people, which the home data suggest to me, then probably they aren't carrying fluoroquinolone-resistant campylobacters either. That is just a commonsense step, and I know that common sense has notoriously misled people before. I think it is common sense that if you look at chickens, you see *Campylobacter*. Some percentage will survive to infect people, and some people will get sick because of it, but I don't find a shred of evidence that this actually happens.

M. Ginevan: One thing we can do is to construct positive models, to see, first, what they tell us, and second, what the data gaps are. The one thing that I don't think we will be able to do, certainly with the available data, is to tell whether or not these models represent truth or any close approximation to it. I don't think we are there yet. I don't hear people saying that we know this. I hear people saying that we hear this but it doesn't bear scrutiny, and that what we know isn't what we would like to know. I think the key is to try to develop models, and iterate the development of the models to the point where we have put a lot of factors aside. Is the use of fluoroquinolones in poultry a clear and present danger in terms of *Campylobacter* resistance? There doesn't seem to be any evidence to that effect. As Tony pointed out, the signs in the regression equation are wrong. I think there are many other things out there which we need to look at before we can have any confidence that we know what is going on.

G. Tillotson: Something to keep in mind about fluoroquinolone resistance is that it is not a transferable resistance, as we see with other antibiotics. It is a point mutation in an individual bacterium, and only its daughters are going to remain resistant. It is not transferable as with tetracycline, where resistance can be transferred from *Campylobacter* to *E. coli.*

T. Wassenaar: That remains to be proven, because why could not a point mutation transform from one strain to another? The wheel doesn't have to be reinvented. Successful genes are spreading by transfer within the species, but not every episode of fluoroquinolone resistance arises from a spontaneous point mutation. We don't know to what extent already existing genes are spreading by transformation.

Question: *Is there any evidence to show that there is transferable resistance?*

T. Wassenaar: For the GYR A gene, it has not been shown yet. It is not a difficult experiment to do, and would predict that it will happen, but whether it will happen outside a host gut, which is when mixing of strains that are not resistant occurs, is a different question.

C. Thornsberry: It is not that simple. There has been one legitimate report of plasmid-mediated fluoroquinolone resistance, but to get real clinical resistance in these organisms, there would have to be more than one mutation. Clearly, they could be transformed. The real problem here is not horizontal transfer between the bugs, but horizontal transfer between the animals and the humans. The question that we need to answer is, if we look at this whole business in total with fluoroquinolones, issues have been made with *E. coli*, but I don't think that *E. coli* is a problem at all with fluoroquinolone resistance. I think that the 3% or 4% resistance that we find in humans is a matter of infection control. I think that *Campylobacter* is a different story. It seems clear to me that this is one of the cases where,

without doubt, *Campylobacter* will become resistant to fluoroquinolones. If we want to follow up on *Campylobacter* and know the real reason, that is the question we need to answer. Why are these organisms so much more likely to become resistant than other organisms that we see in the gut?

Comment: I have a few comments on that. One is that I think there are different degrees of resistance. There is something that puzzles me, and I would be delighted if somebody here knows the answer. There was an abstract by Laura Piddock years ago, reporting that about 39 of 40 people with resistant isolates responded to treatment with fluoroquinolones, and I have never known what that abstract meant. On the face of it, it sounds like what is resistant in a Petri dish may not be clinically resistant.

G. Tillotson: This could be the case, but I would have to ask Laura to be sure. At one point in time, I think it was the public health laboratory that was defining resistance as something quite different from what we normally consider it to be. They were saying that if the MIC is above 0.1 mg/L it is resistant, and this might be what Laura was talking about.

P. Fedorka-Cray: Maybe I can add to that. Even the CDC case–control study, which used the 4 mg/mL breakpoint, showed that resistant infections responded to treatment. We need to call into question what the definition of resistance is in all these studies; it is not an NCCLS-established breakpoint.

G. Tillotson: I think their point was not that they were calling these clinically resistant, but that there was increasing creeping resistance. I think that is a legitimate thing to do, but the terms clinically susceptible and clinically resistant should not be used.

R. Carnevale: The other big issue that we started to discuss was the theoretical possibility of transfer. Previously CVM predicted resistance emergence once we introduced enrofloxacin. Resistance among human isolates of Campylobacter has allegedly increased since 1996, and it may be related to the consumption of enrofloxacin treated chickens. When I look at the data, I don't see evidence that this is true. There is much evidence that the reality is quite different, which I think is exciting and which I would like to pursue. I would be very curious whether anybody else has obtained data that confirms this. Is the simple story that CVM told correct? When I look at the data and come up with ideas, I live in perpetual fear of being considered eccentric.

O. Radostits: Could I ask Clyde to comment on the statement you made that fluoroquinolone resistance is inevitable? What are the potential consequences of that happening in the context of this symposium today?

C. Thornsberry: That wasn't quite what I said. What I said was that, compared to *E. coli*, resistance to a fluoroquinolone is much more likely to develop in a campylobacter, I don't know the reason for that.

T. Wassenaar: One of the reasons may be that *Campylobacter* can become resistant through a one-point mutation. Another thing is that the cost of being resistant for a campylobacter is probably very low, and this has severe consequences. If we were to remove the selective pressure caused by the antibiotic, it might not lead to much change in the population at shorter or even medium periods of time. In other words it doesn't matter to the organism whether it has the mutation GyrA or not; it will grow equally well. Even if the selective pressure is removed, the problem will not be solved.

D. Newell: I am not sure that this is necessarily true; 1% of flocks in the USA are being treated with fluoroquinolones, but we are not seeing a huge number of fluoroquinolone-resistant campylobacters, and I think that there is a cost for a gyrase mutation. We just don't know what it is at the moment, and studies are urgently needed in this area.

G. Tillotson: I would like to add something to what Tony Cox was asking before. Fluoroquinolone resistance is supposedly growing on a daily basis, and we are actually seeing a lot more chicken consumption in this country. From what I understand, it has increased by 25–33% in the last 2–3 years. That in itself should also be leading to an increase in the incidence of *Campylobacter* infections, and also the fluoroquinolone resistance. This is a very complex story, and we don't have definite answers.

D. Meeker: I have a question on the quantitative aspects of poultry fluoroquinolone use and the presence of resistant organisms. If the drug is being used in fewer than 1% of the chicken houses at any one point in time, I don't understand how there can be a 10-fold or 15-fold leap in the incidence of resistant organisms, unless, at the processing stage, there was successful persistence of these organisms that were never cleaned out from shift to shift or day to day. It seems that, on a micro-scale, by following one cohort of treated birds through their state of processing and seeing what happens to that production house with the next cycle or the next cycle when antibiotic was not being administered, you would get a sense of how persistent the blip is coming from that one source and that one processing plant. To me, it seems that this question could be addressed.

C. Hofacre: That is the exact same question that poultry veterinarians have asked; if the resistance was due to our use of enrofloxacin, and such a small amount was used, why is the level rising at the rate that the CDC says that it is rising?

M. Ginevan: Has anyone related it to air travel?

T. Cox: This is a useful thing to look at, and, actually, if foreign travel is subtracted out, it is my impression

that the *Campylobacter* fluoroquinolone resistance rate in the USA has not been increasing. I therefore think that travel is a significant factor.

M. Kist: I have an example from the European countries. If it is true that this incidence of fluoroquinolone-resistant *Campylobacter* strains in humans results from eating poultry, then it is difficult to understand why, for example, in the UK 12% of strains isolated from poultry are resistant, and in Spain 100% are resistant. Also, if we look at the figures for resistance in humans, we see resistance rates of about 15% in the UK for human strains, but 80% in Spain. I don't think that there are large differences in the use of fluoroquinolones in poultry flocks between Spain and the UK. There must be other factors leading to these different levels of resistance in different countries. One of these could be the use of fluoroquinolones in human medicine. To my knowledge, fluoroquinolones are used more extensively in Spain for the treatment of human infections in comparison to other countries. They are also used prophylactically in Spain.

D. Newell: Is it not also true that travelers who leave the USA use ciprofloxacin prophylactically if they go to Asia or Mexico?

M. Pasternack: Yes.

D. Newell: So, all those travel-related campylobacters have to be resistant.

C. Thornsberry: Travellers may not be the only source. In the USA, about $1 billion worth of ciprofloxacin is used every year, and about $1 billion worth of levofloxacin, plus ofloxacin and other drugs. If you try to base resistance on the usage of drugs, you really have to stretch a point. There are essentially no data supporting the idea that drug usage causes resistance. I get frustrated when I am reviewing manuscripts. The writers make these claims, but don't supply any references. I am not sure how much effect the use of drugs has on the development of resistance. I think that what we need to do is to match the drug and the bug. With fluoroquinolones, *Campylobacter* is one of those bugs that does become resistant, but from what you were talking about today, given the right conditions, it grows rapidly. Maybe some selective pressure is created. I think that there is more to consider here than the amount of drug that we use.

Another point that we should make is that antibiotics don't cause resistance, but select resistant strains; this is not always realized by the community.

R. Carnevale: Is there any scientific evidence that the use of fluoroquinolones in poultry has any beneficial effect on human populations, by providing more wholesome or cleaner food?

M. Ginevan: That is an interesting question, and the answer depends on who you ask. According to the USDA data, the incidence of salmonellosis in inspected poultry has been dropping over the last several years. This may or may not be connected with fluoroquinolone usage; hopefully, it has more to do with the HACCP program, but this is going to be difficult to evaluate, because the situation is not very static. The inspection systems and the processing procedures keep changing.

E. Gonder: I have heard it said that the use of enrofloxacin strengthens the intestine of birds. I have also heard it said that enrofloxacin weakens the intestine of birds, and that unhealthy birds that go to market may be smaller than average, and more likely to be cut up in the process and to spray their fecal contents and gut matter around the processing plant. If some of this is true, and if the ban on enrofloxacin would lead to smaller birds that are not calibrated with respect to current equipment, and if chickens are the problem, which is a key 'if', I can see how a higher microbial load might reach the consumer. However, I have not seen any data to indicate whether this is true or false.

C. Hofacre: It is difficult for me to comment on that, except anecdotally. I think it would be reasonably safe to say that if you have sick birds and you send them into the processing plant, generally the intestines tend to be somewhat more fragile, and you experience more fecal contamination during the entire procedure, leading to an increased risk. If you have a flock of birds which is sick for almost any reason, and the sickness begins to affect the growth rate, this does not occur uniformly within the flock. Consequently, the uniformity of the flock decreases with respect to whether they are sick and treated or sick and not treated. This has a negative impact, particularly on chicken plants where a high proportion of the equipment is highly automated and they are operating under very tight size criteria. This is less of a problem for turkey plants, where we can tolerate more changes and differences in size, and where the procedure is considerably less automated.

T. Cox: I haven't seen any studies connecting gut fragility with fluoroquinolone use or non-use. However, I have seen studies that have associated feed-grade antibiotics to prevent necrotic enteritis with a healthier gut that does not rupture during slaughter. The major use of fluoroquinolones in poultry is probably not going to be for intestinal disease.

D. Newall: Does anybody have any data on the incidence of fluoroquinolone resistance in organically kept birds that are not themselves treated?

T. Cox: I have tangentially relevant data, from Effler's data set from Hawaii. It turns out that, in some subsets, there is a variable called organic prod, which is organic produce, but it does not comprise chickens. This appears to be a risk factor for campylobacteriosis.

D. Meeker: There was an article some years ago describing fluoroquinolone-resistant bacteria in bustards in Saudi Arabia.

R. Carnevale: I would like to get this group to give some sort of view on this. I have been looking at this issue for some years now, and I don't think that anyone can argue that the data are clear-cut one way or the other. Clearly, using antibiotics selects for resistance. Clearly, people can get *Campylobacter* infections from a number of sources, and poultry probably comprises one of these. The question is, what is the real level of risk? This is really what we are dealing with here. We are not dealing with theoretical arguments about whether it can happen, but with arguments about what is the significance of that risk, and what is the significance of the potential transfer of these resistant bacteria, because we have to consider the remedies being proposed, both by activist groups and by government agencies; that is, total and unequivocal bans. These are products, in the case of fluoroquinolones in this country at least, that are highly regulated, prescription-only drugs. They are restricted to veterinary use for therapeutic use only, but it seems to me that the question is whether the data justify taking the kind of risk management approach that is being suggested, which is complete elimination, as opposed to more judicious use, which is already being practiced. The level of use in the USA is maybe 1% of flocks, and I think that we need to get some opinions on whether the risk is high enough to justify what is being proposed. In my opinion, it is not; but then again, we are operating in an arena of zero-risk policy. If we are going to take that approach, it jeopardizes the use of many things, whether they be for humans or animals.

O. Radostits: I was going to ask the same question, because it seems to me that sooner or later we will be able to demonstrate that a *Campylobacter* infection in a human came from a chicken, from a cow, or from a horse. It has to happen sooner or later. It seems to me that decisions are being made based on one, two or three cases. If it happens once, it is going to become an avalanche, and regardless of what this group says, or any other group says, in the end what matters is what the FDA says. How can we go to the FDA and point out that decisions should not be made based on one or two cases?

T. Cox: I have heard the CVM say in a public meeting that it is concerned about the avalanche theory, which is why we may not be seeing much now. However, I think that, because of the way in which they do their attributable risk calculations, they think that there is a big effect right now. There are 11 000 people with excess days of diarrhea that could be prevented. I think that this is based on a miscalculation, but that the CVM is concerned that this is the tip of the iceberg. I have heard many people around this table ask whether it is a good idea to remove enrofloxacin, and whether anyone thinks that it would prevent a detectable number of human illnesses; and I haven't heard anyone say that they are sure that this is true. I feel that if we had the CVM with us today, we might possibly hear a different response. The CVM thinks that the data show that this is true; my understanding is that the CVM partly looks at the trends, does not correct those trends for travel, and if it finds a trend that is upward, then it decides that maybe some fraction of that is attributable to enrofloxacin use. This seems to me to be a very confused line of thought, but that is where regulatory policies take us. I would like to know whether anyone has confidence in the evidence that there is a detectable causal link between enrofloxacin use in chickens and resistant organisms in people. I would even generalize that, and ask what fraction of all *Campylobacter* cases is clearly attributable to chickens as opposed to something else, or not attributable at all? I know what my data tell me.

O. Radostits: What kind of evidence do we need to support the causal link?

M. Ginevan: If, in different parts of the country, or in different countries, where there are strong seasonal fluctuations in chicken-driven campylobacteriosis and resistance, it was observed that human populations somehow consistently echoed what was going on in the chicken population, maybe with some lag time between them, that to me would be very impressive evidence; however, the seasonality doesn't work out right as far as I can tell. That is one kind of evidence. Another kind of evidence might be if international data were examined, with correction for travel, there was no increase in resistance in human pathogens before enrofloxacin was introduced into a country. For example, in 1970, ciprofloxacin could be introduced, without an effect on resistance. In 1980, enrofloxacin could be introduced, and an increase in resistance or a consistent pattern could be seen. I would suggest that this would be evidence for a causal connection. From what I have been able to tease out of international data, which is confusingly and distressingly sparse, it looks as though trends don't have much to do with when enrofloxacin was introduced into different countries. These are possible lines of argument that could support a causal connection. I would welcome other ideas and suggestions on what are the testable consequences of a causal connection.

D. Newell: We have recently reviewed the seasonality data across Europe and the matching data for chickens and humans, and, if anything, the data from many countries suggest that humans give *Campylobacter* to chickens, not the other way around. This is much more consistent with there being some sort of common source for both when it comes to seasonal peaks.

E. Gonder: I think it may be possible to make some scientific conjectures, at least that there is a weak link between fluoroquinolone resistance in chickens and fluoroquinolone resistance in humans. The problem we are going to be faced with is the one that I am sure the CVM worries about, which is what is an acceptable

degree of risk? I haven't seen much progress on its part in trying to decide whether 5000 people with diarrhea are worth 300 000 dead chickens and some environmental damage. I am not sure if the CVM has any idea what constitutes an acceptable degree of risk, and I think that the chances of it making that decision itself are remote, because it is a political decision. Consequently, while we need the science, we also need to move the entire conversation into a different area that addresses welfare and environmental concerns as well as strictly public health concerns.

M. Ginevan: When you mention environmental concerns, are you are referring to the removal of diseased carcasses?

E. Gonder: I am referring to the fact that it takes 2 lb of feed to produce 1 lb of chicken. If the chicken dies, that feed is wasted. You then have a consequent deposition into the environment of nitrogen and phosphorus. We also have to consider the other environmental resources dedicated to the milling, polling and eventual removal of that bird, and the additional crop space that would be required to take care of it.

M. Ginevan: It could also be that there are 10^{10} bacteria per gram of stool if the dead bird is being buried, and source water contamination by all the carcasses of the birds that are being buried.

E. Gonder: Actually, most of them are being rendered.

T. Cox: Kim Thompson mentioned the idea that what is acceptable depends on the cost. Whether what is going on now is acceptable is partly a matter of balancing risk and cost benefits, but there is also a prior question: is there in fact any detectable risk, and is it acceptable? From a cause and effect point of view, the more interesting question is whether there are detectable risks at present. Are our chickens making people sick, and are they making them sick in a fluoroquinolone-resistant way? That is an interesting sub-question. We don't even have to worry about susceptibility until those questions can be answered.

L. Vogel: The hypothesis is that if somebody is prescribed a fluoroquinolone as empirical treatment, and they have been infected by a fluoroquinolone-resistant campylobacter, then the treatment won't work, and there will be 6 extra days of diarrhea.

T. Cox: In fact, I think that, in the final risk assessment, the FDA/CVM didn't state the number of days of diarrhea, but just said that there would be an adverse health effect. They didn't say what that health effect would be.

L. Vogel: They consider being prescribed a fluoroquinolone while carrying a fluoroquinolone-resistant bacterium to be a bad outcome in itself, whether or not the bug responds to treatment or whether or not there are clinical sequelae.

T. Cox: If there is no evidence that fluoroquinolones have much effect on excretion anyway, how can you make these assumptions?

L. Vogel: It appears that the occurrence of a single case is not worth any potential benefits, thus unless withdrawal has negative consequences, it is desirable.

T. Cox: To the extent that size homogeneity is destroyed and greater variation is introduced into the process, if it is true that chickens carry microbial loads to people, then almost certainly it is the unusual chicken with the high microbial load that counts, not the average chicken flowing through the process. To the extent that you take action that increases the variability of the processing, and, arguably or obviously, banning fluoroquinolones would tend to do that, you may actually create an adverse effect where there isn't one now. I think the question of what will happen to humans needs to be thought out quite carefully. The FDA approach, and I think this is what Kim Thompson was getting at with the decision analytic context for the risk analysis, is to say that we are going to calculate attributable risk, and when that proportional attributable risk gets too high, we are going to intervene to protect public health; however, the FDA never says what would be the consequences of that intervention, and whether it will make things worse than they already are.

R. Carnevale: I think that what you will also find is that the FDA will say that it is a public health agency, so animal health is not an issue. Also, it will say that while fecal contamination may create some bacterial contamination of carcasses, that is not an FDA issue, but a USDA issue, so it is not their problem.

M. Ginevan: At slaughter facilities, what level of cleanliness, surveillance and microbiological assessment is carried out, on a routine day-by-day basis?

E. Gonder: This varies somewhat from plant to plant. The government requirements concerning bacterial monitoring are really not very well thought out or particularly effective. All plants are cleaned from top to bottom once a day. For example, chillers are drained, completely sanitized, swept down, hosed down, and disinfected, and equipment is disassembled. Different plants approach bacterial monitoring in different ways. The particular one that I am involved with monitors chiller water, surface contamination and number of bacteria per gram in different products, and does skin swabs for *Salmonella* and *E. coli* on a fairly regular basis. USDA bacterial monitoring or the required bacterial monitoring is inadequate. You probably saw the *Salmonella* performance standards on turkeys, where the Center for Science in the Public Interest, under the Freedom of Information Act request, named the 'filthy

five' turkey plants. This ranking was based on 55 individual bird samples per plant. Thus we have the USDA releasing information to a consumer group for a plant that may process, say, 70 000 birds a day, and an annual ranking based on a sample of 56 birds, and the USDA contending that this is a statistically valid process control.